The striated muscle myosin coiled coil is known to contain regional variations in its functions, such as the polymerization competent light meromyosin (LMM) and the flexible hinge regions. Variations in the structural stability of the coiled coil are an integral part of these functions. To ascertain the variability in stability along the myosin coiled coil, four different regions were analyzed using Free Fall Force Spectroscopy to reversibly pull the two α-helices apart in each location. The targeted regions analyzed were the LMM, and two S2 locations just C-terminal to the S1/S2 hinge defined by site-specific antibodies. Additionally, the S1/S2 hinge was tested by exploiting the rigor bond to impart forces N-terminally to its hinge. As expected, the S1/S2 hinge reversibly unraveled to the greatest extent under less force than the other regions. Regions more C-terminal to the S1/S2 hinge required increasing amounts of force to unravel, and the LMM was the most rigid. A plausible explanation for the variations in stability is that the coiled coil can be pulled apart more readily near its end. In particular, it is observed that regions furthest from a terminus require a high amount of initial force application to separate compared end regions. However, as the strands begin to separate at a site close to the N-terminus, the force-distance curve shapes suggest that the N-terminal part of the coiled coil has completely unraveled and only the C-terminal coiled coil continues to provide resistance. By using the Free Fall Force Spectroscopy, these fine mechanics can be investigated with piconewton to subpiconewton amounts of applied force. It also demonstrates reversibility of the unraveling under repeated cycles of force application on a single molecule. (sponsored by NSF ARRA)

193-Plat
A Novel Mouse Model of Nebulin-Based Nemaline Myopathy
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Nebulin - a giant sarcomeric protein - plays a pivotal role in skeletal muscle contractility by regulating thin filament length and function. Although mutations in the gene encoding nebulin (*NEB*) are a frequent cause of nemaline myopathy (NM), the most common non-dystrophic congenital myopathy, the mechanisms by which mutations in *NEB* cause muscle weakness remain largely unknown. To better understand these mechanisms, we have generated a mouse model in which *NEB* exon 55 is deleted (NEBΔex55), a mutation known to frequently occur in NM patients.
NEBΔex55 mice are born close to Mendelian ratio's, but show growth retardation after birth. Electronmicroscopy shows nemaline rods - a hallmark feature of NM - in muscle fibers from NEBΔex55 mice. Western blotting studies with nebulin-specific antibodies reveal much reduced nebulin levels in muscle from NEBΔex55 mice. Immunofluorescence confocal microscopy studies with tropomodulin antibodies and phalloidin reveal that thin filament length is reduced in muscle fibers from NEBΔex55 mice. In line with reduced thin filament length, the maximal force generating capacity of skinned muscle fibers is reduced in NEBΔex55 mice with a more pronounced reduction at longer sarcomere lengths. Finally, in NEBΔex55 mice the regulation of contraction is impaired, as evidenced by marked changes in cross bridge cycling kinetics and by a reduction of the calcium sensitivity of force generation. This reduced calcium sensitivity was observed only at short sarcomere lengths, suggesting that nebulin might play a role in the length dependence of activation.
In conclusion, we have generated the first nebulin-based NM model. Our data indicate that the phenotype of NEBΔex55 mice closely recapitulates that observed previously by us in patients harboring this particular mutation.

Platform: Chromatin & the Nucleoid

194-Plat
Nucleosome Dynamics Studied by Single-Pair FRET and Computer Simulations
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DNA in nucleosomes is sterically occluded and nucleosomes must open to allow full DNA access. We studied this process by single pair FRET (spFRET), all-atom and coarse-grained molecular dynamics. spFRET shows evidence for a new structural intermediate preceding histone dissociation, in which the (H3-H4)2 tetramer/(H2A-H2B) dimer interface is split open. This is followed by H2A-H2B dimer release from the DNA and, lastly, (H3-H4)2 tetramer removal. This open intermediate state could be demonstrated in Xenopus and yeast nucleosomes; histone variants such as H2A.Z change the mechanism of opening. We estimate that the open state is populated at 0.2 - 3 % under physiological conditions, and could have significant in vivo implications for factor-mediated histone exchange and for DNA accessibility.
To look for subpopulations and changes in FRET distances during the salt-induced transition for the various FRET pairs, we used photon distribution analysis (PDA). We observed two to three populations for all the FRET pairs, their calculated distance corresponding to the values expected from the dye positions in the crystal structure. Histone tail acetylation increased these distances and shifted the midpoint of the opening transition to lower salt concentrations.
The effect of histone tail modification was studied by all-atom MD simulations with 100 ns trajectories of the full nucleosome. We find evidence for an internal allosteric transition at the H2A-H3 interface induced by the removal of either the H3 or the H2A N-terminal tail. DNA dynamics on the histone core are studied by a new coarse-grained model; these simulations show a strong influence of the presence of the histone tails on DNA unwrapping, and an open state that can be stabilized by the displacement of the H3 tail into the gap between the DNA and the histone core.

195-Plat
Kinetics and Thermodynamics of Nucleosome Winding and Unwinding
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Nucleosome post-translational modifications (PTMs), histone variants, and co-factors can activate and repress transcription of the DNA they package. Modified and variant nucleosomes are thought to regulate transcription by two mechanisms acting independently or in concert: ''Tuning'' the DNA binding affinity, or recruiting additional cofactors. To truly understand the regulation of nucleosome packaging processes, it is essential to deconvolute the effects of cofactor recruitment from the effects of changes in binding affinity. To distinguish these two mechanisms, we use single molecule optical trap measurements of nucleosomes under force to measure the kinetics of transitions between nucleosome states. Using a system of known phenotypic response (the sin mutant nucleosomes) we find that nucleosome variations affect the transition rates between states of nucleosome unwinding. From these rates, we are able to determine the free energy difference induced by a nucleosome variation, thus correlating a phenotypic response with rates and energy and giving mechanistic meaning to chromatin ''loosening''. Our measurement of the energy serves as a benchmark against which the kinetics and free energies of nucleosomes containing histones with PTMs, variants, or cofactors may be compared to in future studies.

196-Plat
NAP1-Assisted Nucleosome Assembly on DNA Measured in Real Time by Single-Molecule Magnetic Tweezers
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While many proteins are involved in the assembly and (re)positioning of nucleosomes, the dynamics of protein-assisted nucleosome formation are not well understood. We study nucleosome assembly mediated by the chaperone NAP1 at a single-molecule level in real time with magnetic tweezers. We show that the number of assembled nucleosomes on each of the DNA molecules can be estimated based on the change in supercoiling density and end-to-end length. Furthermore, we demonstrate that this number can be verified by observing the number of disassembly steps when applying high force. With our experimental conditions, we assembled up to ~20 nucleosomes on 8 kb long dsDNA within ~300 seconds. Once the nucleosomes are formed, they are stable, and no disassembly was measured for several hours. Interestingly the association of histones H3 and H4 to the DNA by NAP1 shows a similar DNA compaction compared to nucleosome assembly, but no change in supercoiling density. Histones H2A and H2b with NAP1 do not bind to the DNA.
This data indicate that NAP1-assisted assembly of complete nucleosomes occurs as a two-step process. We suggest that first histones H3 and H4 are associated to the DNA as oligomers, where the DNA is partially wrapped around this substructure, inducing an end-to-end length decrease but no change in linking number. Subsequently, NAP1 brings histones H2A and H2B to the DNA upon which the complete octamer is formed, and the DNA fully wraps around

the histone complex, forming a complete nucleosome and changing the linking number.
Our method allows measuring the number of assembled nucleosomes without disrupting them. All our experiments have been carried out at force and torque conditions resembling those in the nucleus and with natural DNA instead of tandem repeats of strong positioning sequences.

197-Plat
Nucleosome Conformation and the Higher Order Structure of Chromatin: spFRET Experiments on (Di)Nucleosomes
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Chromatin, the structure in which DNA is compacted in eukaryotic cells, plays a key role in DNA regulation by governing DNA accessibility. FRET experiments on single nucleosomes, the basic units in chromatin, have revealed a dynamic nucleosome where spontaneous unwrapping from the ends provides access to the nucleosomal DNA. We investigate how the dynamics of individual nucleosomes couples to chromatin higher order structure. Therefore, we study DNA dynamics in nucleosomes flanked by linker DNA or a second nucleosome.
A combination of single-pair (sp) FRET, Alternating Laser Excitation and FCS on nucleosomes in gel with increasing linker DNA length reveals that the fraction of partially unwrapped nucleosomes increases when the exiting DNA tails come close to each other. Electrostatic repulsion between the entering and exiting DNA drives nucleosomes to a more open conformation.
Nucleosome-nucleosome interactions further affect DNA unwrapping. A stacking interaction, mediated by histone tails, is expected to bring nucleosomes face to face in order to establish the structure of a folded chromatin fiber. Dinucleosomes with 20, 50 or 55 bp linker DNA adopt a conformation similar to mononucleosomes, as measured with gel electrophoresis and bulk FRET. However, spFRET experiments show that the fraction of partially unwrapped nucleosomes increases from 15% to 25% for dinucleosomes with 55 bp linker DNA as compared to 50 bp and to mononucleosomes. We propose that this additional unwrapping helps to reduce the energy penalty for bending the linker DNA in stacked nucleosomes.
These spFRET experiments reveal subtle changes in nucleosome conformation that form the basis to higher order chromatin structure and function.

198-Plat
Effects of DNA Methylation Patterns on Chromatin Compaction
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Gene regulation in eukaryotic cells is modulated by epigenetic modifications. DNA CpG methylation is the most common epigenetic modification found in DNA. Although DNA methylation has been generally affiliated with chromatin compaction and gene silencing, the effect of different DNA methylation patterns on the chromosome conformation remains elusive. In this study, we evaluated the effect of DNA methylation level and DNA methylation patterns on the dynamic conformations of DNA, nucleosomes and nucleosome arrays. Changes in the nucleosome conformation are monitored using time-resolved fluorescence spectroscopy coupled with FRET. The compaction and the self-association pattern of nucleosome arrays are examined using Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogram (PCH). Our results suggest that nucleosome and nucleosome array conformations are distinctively modulated by DNA methylation patterns arising from their relative location within a nucleosome. The binding affinity of a histone octamer to DNA can also be altered by different DNA methylation patterns. In addition, DNA methylation is found to play an important role in mediating the self-association between nucleosomes and nucleosomal arrays. The implications of these findings in chromatin organization and gene regulation will be discussed. These findings are expected to elucidate the molecular mechanism of DNA methylation in regulating gene silencing events.

199-Plat
The Synergy Between DNA and Nucleosomes in Chromatin
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How the genome accommodates the tight packing needed to fit in the cell nucleus and at the same time maintains the accessibility necessary for specific expression is one of the open questions in modern biology. In eukaryotic cells DNA is wrapped and packaged into chromatin through the binding of histones and formation of nucleosomes. In addition to bundling DNA, the nucleosomes also facilitate communication between distant genomic sites, such as enhancers and promoters found at the ends of protein-mediated loops. In order to understand such processes, we have begun to investigate chromatin organization and looping. We have developed a mesoscale model of chromatin at a resolution of a single base pair and used Monte Carlo numerical strategies to unravel how the presence of nucleosomes on DNA can influence and possibly control chromatin looping. We have validated this model by successfully reproducing experimental measurements of gene expression on nucleosomal arrays. Our results show a wide variety of chromatin organization depending on the way nucleosomes are positioned on DNA and also on chemical details at the histone level, such as modifications of the N-terminal tails. This diversity in chromatin organization, which extends beyond the conventional solenoid and zigzag models, comes along with very different physical and mechanical properties and looping propensities. Furthermore, our simulations reveal some surprising properties of chromatin: for example, we found that tightly packed chromatin fragments are the most flexible, a remarkable feature for a material that needs to fit inside a cell nucleus. In conclusion, our work uncovers parts of a rich and dynamic picture of chromatin where the DNA sequence influences the positioning of nucleosomes, which in turn shapes the genomic material and has an impact on the accessibility and expression of the genetic message.

200-Plat
Single-Molecule Studies of Chromosome Organization by SMC
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The structural maintenance of chromosomes complex (SMC) plays an essential role in chromosome condensation, sister chromatid cohesion, and recombinational repair. SMCs possess a unique structural architecture: SMC monomers dimerize through interactions within their hinge domains while long coiled-coil arms bring together the N- and C-termini which form an ATPase head domain. Despite its cellular importance and extensive efforts, the role of the ATPase domain of SMC still remains mysterious. How ATP binding and hydrolysis is coupled to DNA remodeling or how the mechanochemical cycle of SMC modulates the SMC-DNA interaction is unclear. Using Bacillus Subtilis SMC (BsSMC) as a model we are utilizing single-molecule microscopy to visualize how SMCs interact with flow stretched DNAs. In an approach we have termed DNA motion capture, we site-specifically label DNAs with quantum dots which allow us to track how DNA segments under differing tensions are condensed by SMC. We find that BsSMC is able to compact individual DNAs even in the absence of ATP although ATP greatly enhances the rate of DNA compaction. This supports a picture in which ATP does not provide a powerstroke for DNA organization but instead acts to bias BsSMC conformations that facilitate chromosome remodeling. To better understand the role of ATP in the mechanochemistry of BsSMC we examined how two mutants, E1118Q which slows the rate of ATP hydrolysis and S1090R which blocks head-head engagement, alter the kinetics of BsSMC-mediated DNA condensation. Our single-molecule assay shows that the E1118Q mutant compacts DNA more slowly than the wild-type in the presence of ATP, and significant compaction rate reduction was observed with S1090R mutant. These results suggest a role for both ATP hydrolysis and SMC head reengagement in SMC-mediated DNA condensation.

201-Plat
Tracking the Location and Dynamics of Chromosomal Loci in E. Coli
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We have used widefield fluorescence microscopy to locate fluorescently labeled, specific DNA loci on the *E. coli* chromosome. Their cellular position, short and long-time motion were tracked with ~30 nm accuracy. In addition to the conventional analysis of the location of the loci in the long axis of the cell, we have also analyzed their locations along the short axis. The origin of replication (*oriC*) has been postulated to be anchored to the cell membrane, which helps regulate the initiation of replication. In slow growth conditions the distributions of *oriC* and the other tested loci (including the *rrn* operon *rrnD*) in the short axis are peaked in the center. In moderate growth conditions they are peaked 200-300 nm away from the cell center. We examined the extent to which loci sample the entire nucleoid on a short timescale (1-3 s). Dynamics of each locus were studied for several hundred cells by tracking their position for several seconds. Similar to the other tested loci, in MBM *oriC* undergoes confined diffusion ($D_1 = 9.7 \times 10^{-3}$ $\mu m^2 s^{-1}$) in a ~100 nm domain that itself diffuses further on a longer time scale ($D_2 = 7.8 \times 10^{-4}$ $\mu m^2 s^{-1}$). Foci were also tracked over the entire growth cycle. Periodic motion was observed in the short axis suggesting the DNA rotates as it segregates. The cytoskeletal